

Valve Ultrastructure of Some Eunotiaceae (Bacillariophyceae), with Comments on the Evolution of the Raphe System

by

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MAR 07 2000

Ultrastructural observations on valve features of five diatom species from the family Eunotiaceae are presented, including four from the Amazon basin and one from California. Of the Amazonian taxa, *Peronia brasiliensis* Hustedt has a straight raphe system positioned on the valve face and a central sternum, *Eunotia synedraeformis* Hustedt and *E. curvula* Hustedt have raphe systems predominantly towards the valve mantle, as well as a central sternum. *Eunotia conversla* Hustedt has a raphe system positioned more on the valve face and a central sternum. Specimens of *Amphicampa eruca* Ehrenberg from California have a very short raphe on the mantle and lack a central sternum. These data, in addition to previously published observations, point to the diversity of raphe types in the Eunotiaceae. These data do not help to distinguish between differing hypotheses on the length and position of the raphe in the earliest raphe-bearing diatom.

The family Eunotiaceae was originally proposed by Kützing (1844:32) for those diatoms asymmetrical about the longitudinal axis, with convex dorsal and concave ventral margins, and striae extending across the entire face of the valve. Today, we understand the family to contain those raphid diatoms that also possess rimoportulae (Krammer and Lange-Bertalot 1991). The family is almost exclusively freshwater. Included in the group are *Eunotia* Ehrenberg (which, in a broad sense, also includes species formerly separated into the genera *Semiorbis* Patrick and *Desmogonium*), *Actinella* Lewis, *Peronia* Brébisson and Arnott, and *Amphicampa* Ehrenberg. *Peronia* has been reassigned to a new family in which it is the only member (Round et al. 1990), but it is clearly allied to the genera mentioned above. Species of the Eunotiaceae are most diverse in dystrophic or acid waters.

Members of the Eunotiaceae are thought to have a unique systematic position, because of their suite of characters, including both primitive (i.e., rimoportulae) and derived (i.e., raphe system) features. Because both primitive and derived features are found in all members of the group (except some highly derived forms that have secondarily lost a raphe system, see Kociolek and Rhode 1998), members of the Eunotiaceae have been thought to represent early archetypes in the development of the raphe system (Berg 1948). Some (e.g., Kolbe 1956; Geissler and Gerloff 1963) have speculated the raphe evolved through enlargement of the rimoportulae along the ventral margin, by way of small raphe-like slits (with taxa like *Amphicampa* proposed as intermediates between araphid and raphid diatoms). Hustedt (1952), in examining a wide range of eunotioid diatoms from the Amazon basin, believed the raphe evolved in a form similar to the genus *Peronia* (i.e., raphe elongated on the valve face but without a central nodule). In Hustedt's view, most of the Eunotiaceae represent a lineage off the main evolutionary track towards the Naviculaceae. Simonsen (1979) aligned the Eunotiaceae with one group of araphid diatoms, off the line in which the naviculacean raphe evolved. One implication of Simonsen's dendrogram is that the raphe in the Eunotiaceae is not homologous with the raphe system of the naviculoids.

In this report, I present light and scanning electron microscope observations on some Eunotiaceae species that have been suggested by Hustedt (1952) and Kolbe (1956) to represent important links in the evolution of the raphe system. Ultrastructural features are described and compared to other members of the family and to other raphid diatom groups.

MATERIALS AND METHODS

Light and scanning electron microscope observations were conducted on several taxa within the Eunotiaceae thought critical to understanding the early evolution of the raphe system. The taxa examined and material on which the observations were made include *Eunotia curvula* Hustedt (samples AM 1027, AM 1028 from the Hustedt Collection), *E. synedraeformis* Hustedt (samples AM 1027, AM 1028 from the Hustedt Collection), *E. conversa* Hustedt (samples AM 1027, AM 1030, AM 2216 from the Hustedt Collection),

Amphicampa eruca Ehrenberg (sample 607663 of the Diatom Collection, CAS) and *Peronia brasiliensis* Hustedt (samples AM 1027, AM 1028, AM 2216 from the Hustedt Collection).

Hustedt material includes:

Sample AM 1027: "Brasilien. Lago Jurucui, an Pflanzen. 25.10.47. Braun, 252."

Sample AM 1028: "Brasilien. Lago Jurucui, Südufer, an Ast. 5.11.47. Braun, 253."

Sample AM 1030: "Brasilien. Lago Jurucui, überschwemmte Campos, an Gr sern.
25.6.48. Braun 2548."

Sample AM 2216: "Igarape do Tendo. Uferschlick. 22.11.47. Braun 209.b."

CAS sample 607663: "USA, California, Mendocino Co., Lake Mendocino, drying inlet to lake. 28 Jun. 1995. coll. J. P. Kociolek."

Material was cleaned in nitric acid, rinsed in distilled water, settled until neutral, and air-dried onto coverslips. Coverslips containing the dried material were attached to aluminum stubs and sputter-coated with approximately 20 nm of gold-palladium. The coated material was viewed on a Hitachi S-520 SEM at an operating voltage of 10 kV.

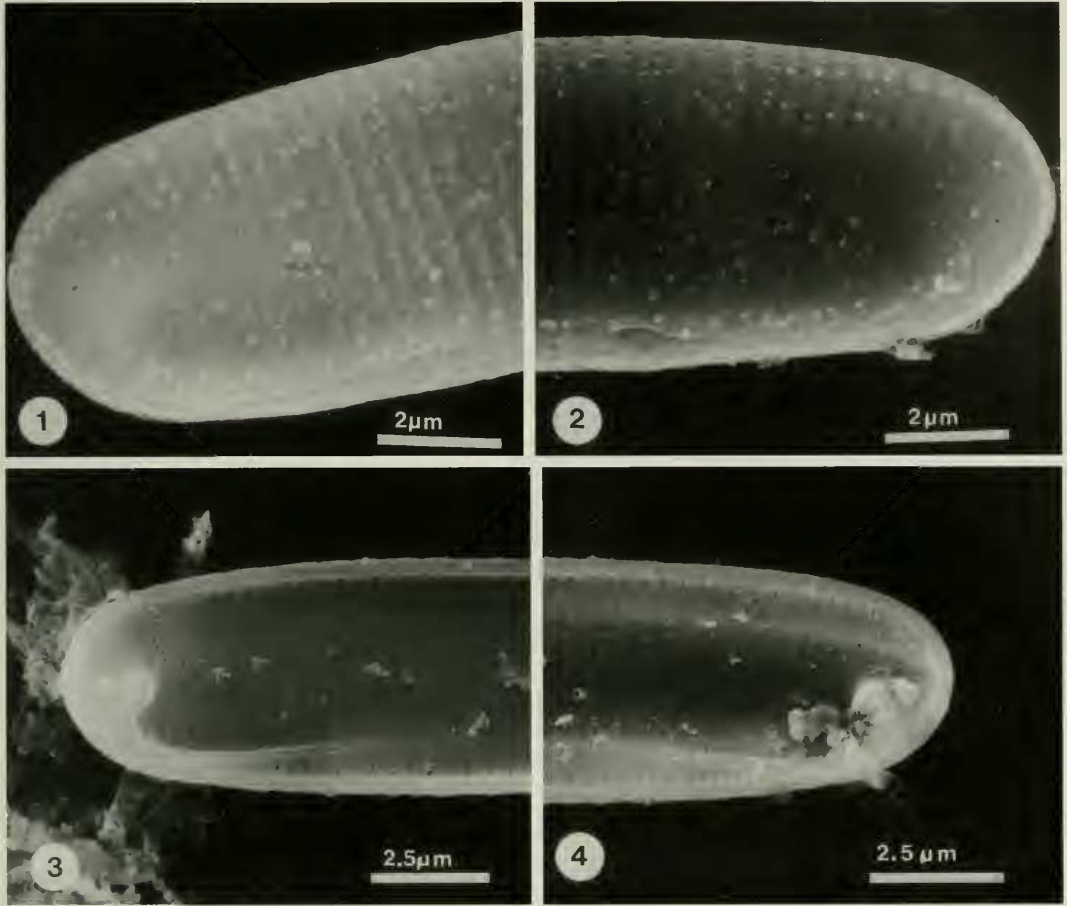
RESULTS

Eunotia curvula Hustedt

Figures 1–4; Hustedt 1952, fig. 24; Simonsen 1987, pl. 568, figs. 1–5

DESCRIPTION. — Valves linear with margins nearly parallel, apices rounded, with convex dorsal margin and concave ventral margin, 150–500 μm long, 4.5–7.0 μm broad. Raphe short, j-shaped, curves from near the mantle onto the valve face, axial area distinct, striae 21/ 10 μm , punctate. Rimoportula present at each valve pole.

SEM OBSERVATIONS. — Round areolae are occluded externally. The raphe is hook- or j-shaped, and has a large dilated distal end and undistinguished proximal end. A single rimoportula opening is found at each valve terminus. The axial area extends the length of the valve. The valve is covered with small siliceous nodules. Internally the raphe slit is bordered on both sides by a small ridge of silica. The raphe runs from the proximal end, positioned on the valve face, towards the mantle, and recurves towards the face, terminating in a prominent helictoglossa. A rimoportula is positioned at the valve face-mantle junction.



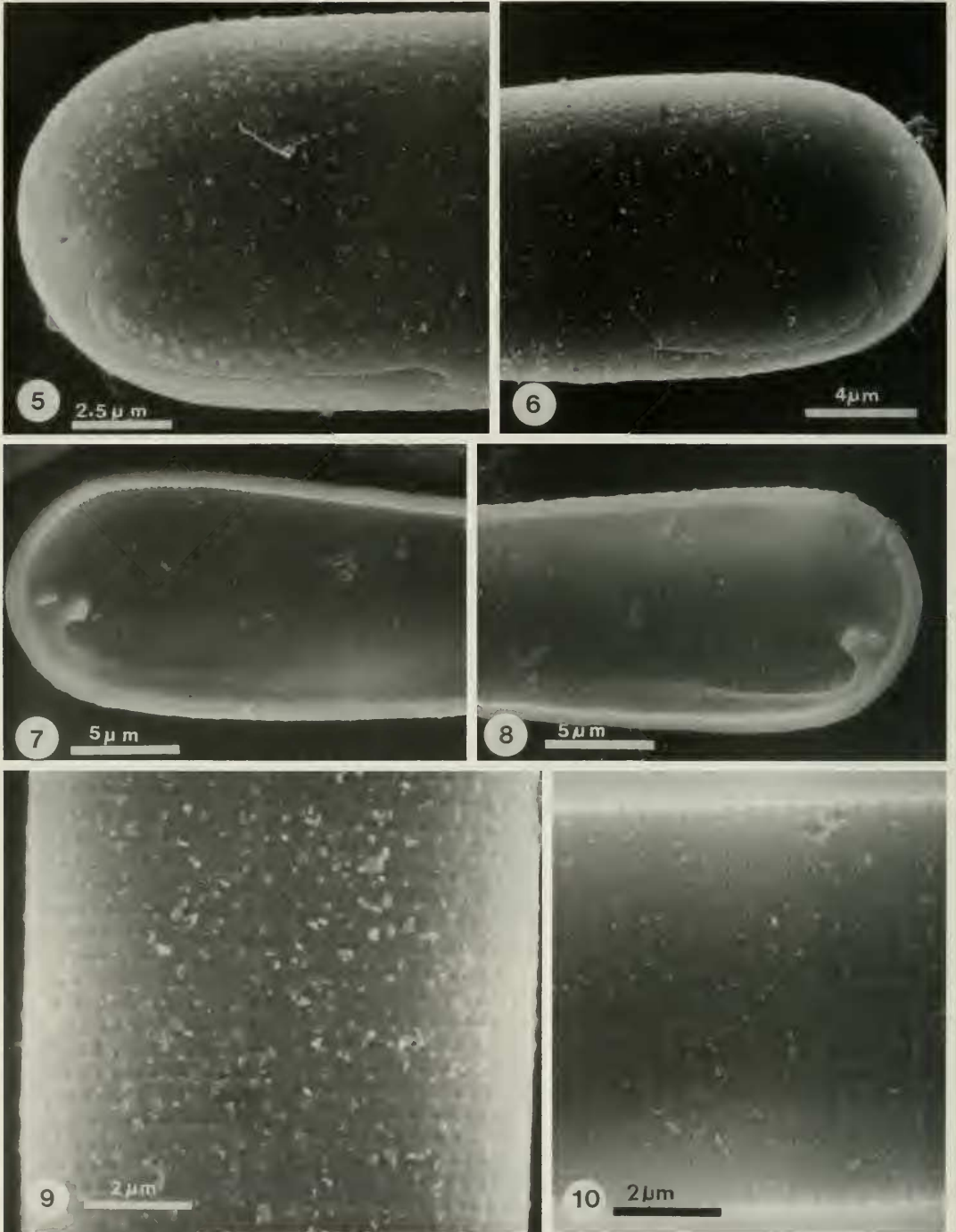
FIGURES 1–4. *Eunotia curvula*, SEM. (1–2) External views at the apices. J-shaped raphe has dilated distal ends terminating on valve face. Rimoportulae are positioned at the valve face-mantle junction. Valve face is covered with scattered siliceous nodules. (3–4) Internal views at the apices. Raphe branches are restricted to ventral margin, with a prominent helictoglossa at each pole. The raphe is bordered by a thin, elevated ridge. Rimoportulae are located near helictoglossa. Striae are interrupted near the center of the valve.

Eunotia synedraeformis Hustedt

Figures 5–10; Hustedt 1952, fig. 8; Simonsen 1987, pl. 566, figs. 1–8

DESCRIPTION. — Valves straight or nearly so, with parallel margins, apices appear slightly swollen and broadly rounded, obtuse, length 235–525 μm, breadth 9–10 μm. Raphe j- or hook-shaped, recurved on the valve face, axial area runs the length of the valve, striae punctate, with puncta near the axial area more coarse and irregularly arranged within the striae. Striae number 18–22/ 10 μm.

SEM OBSERVATIONS. — Rounded to nearly rectangular areolae are occluded externally. The hook-shaped raphe is dilated at both the proximal end (positioned close to the valve mantle) and distal end (positioned in the middle of the valve face). Striae are interrupted along the apical axis by a hyaline area running the length of the valve. A narrowly elliptical, prominent opening of the rimoportula is located at each pole and is positioned at the valve face-mantle junction. Internally, areolae become more distantly spaced as they approach the center of the valve. Occlusions are lacking internally. The raphe slit has a slight ridge bordering it as it runs from the proximal end on the valve



FIGURES 5–10. *Eunotia synedraeformis*, SEM. (5–6) External views at the apices. Prominent J-shaped raphe with dilated distal ends recurved towards center of valve. Rimoportula opening is visible. Occluded areolae appear sunken. (7–8) Internal views at the apices. Raphe opening is restricted to ventral margin, bordered by a thin, elevated ridge, and ends in large helictoglossae. Rimoportulae are found at both poles. (9) Center of the valve. External view showing interruption of striae forming central sternum. (10) Center of the valve. Internal view showing disruption of striae towards center.

face towards the mantle and recurves towards the valve center at the distal end. The raphe terminates in a prominent helictoglossa at its distal end. Located near the helictoglossae at the valve-mantle junction are well-developed rimoportulae.

***Eunotia conversa* Hustedt**

Figures 11–16; Hustedt 1952, fig. 25; Simonsen 1987, pl. 565, figs. 1–8

DESCRIPTION. — Valves narrow, straight for most of the length of the valve, bent about the apical axis, ends narrowly rounded. 50–120 μm long, 2.0–3.5 μm broad. Raphe j-shaped, but difficult to discern. Axial area present but indistinct. Striae about 28–30/10 μm .

SEM OBSERVATIONS. — Internally the raphe slit is located in a raised central rib and extends along the center of the valve. The raphe terminates in a helictoglossa. A rimoportula is found on the face at the valve terminus at only one end of each valve. An internally-raised ridge is found positioned towards the ventral margin of the valve. The ridge extends about 1/3 of the length of the raphe. Striae are interrupted by a wide unornamented axial area near the center of the valve. Externally, the relatively long raphe slit curves and continues back towards the center of the valve. The shorter portion of the raphe branch does not perforate the valve. A prominent opening of the single rimoportula is found at the junction of the valve face and mantle. Areolae appear as small, round openings internally.

***Peronia brasiliensis* Hustedt**

Figures 17–20; Hustedt 1952, figs. 1, 2; Simonsen 1987, pl. 562, figs. 1–7

DESCRIPTION. — Valves narrow, asymmetrical about the transapical axis and have rounded poles. Length 15–30 μm , breadth 3–5 μm . The raphe branches are short, restricted to the apices, and positioned in the middle of the valve face. Striae are parallel, slightly radiate at the poles, 20–30/10 μm .

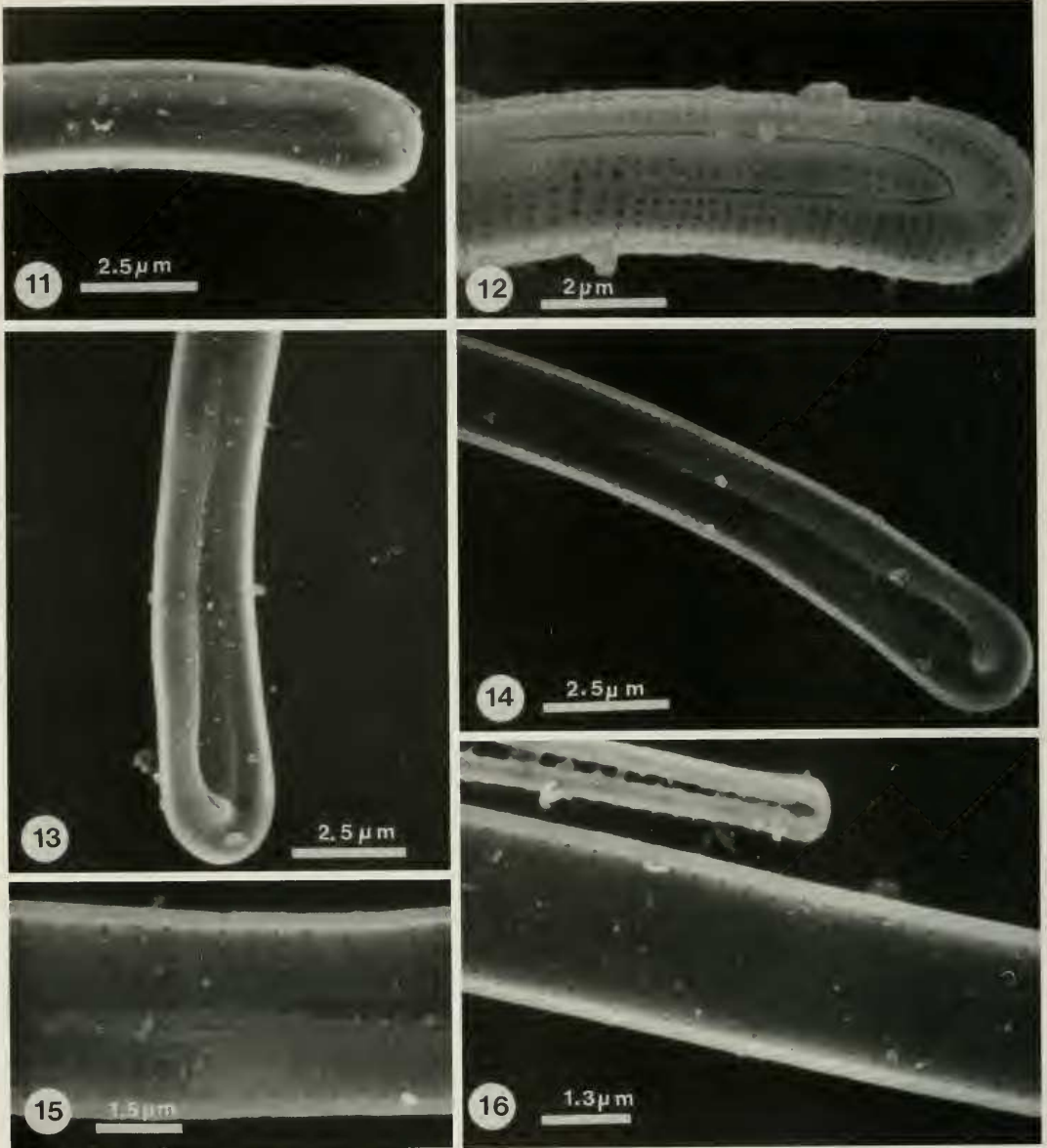
SEM OBSERVATIONS. — Internally, the centrally-positioned, short raphe slits extend from the proximal ends and terminate as prominent helictoglossae. A small, relatively inconspicuous rimoportula is located at one pole only, at the valve terminus. A central nodule is lacking. Between the proximal raphe ends is located an unornamented axial area. The round puncta appear to lack occlusions.

***Amphicampa eruca* Ehrenberg**

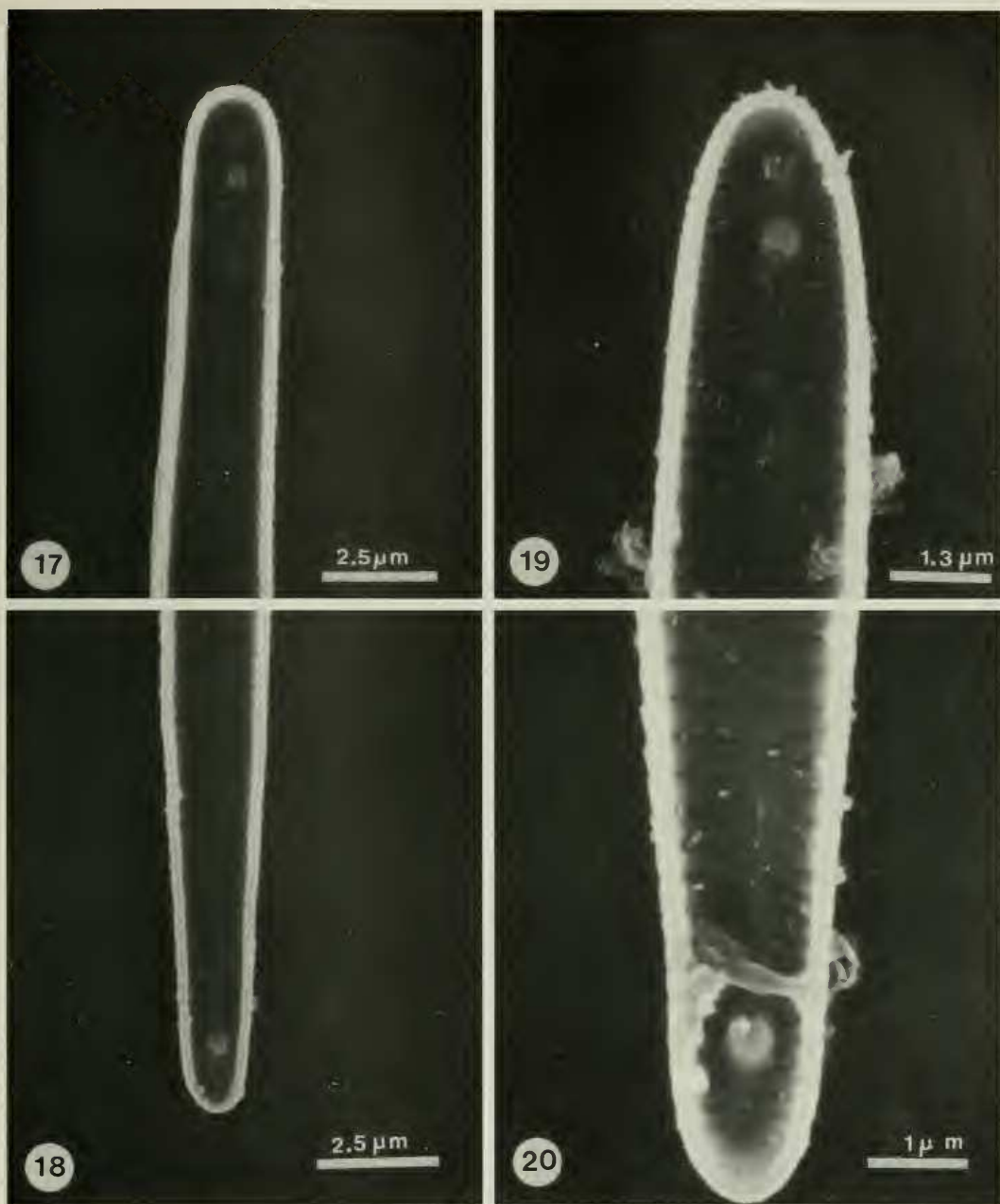
Figures 21–35

DESCRIPTION. — Valves 19–64 μm long, 9–11 μm broad. Both the dorsal and ventral margins are undulate; the dorsal margin has 3–7 undulations, whereas the ventral margin has 1–6 undulations. Striae are distinctly punctate, and the discontinuity across the striae is closer to the ventral margin. Small raphe slits are indistinct but visible near the valve terminus close to the ventral margin.

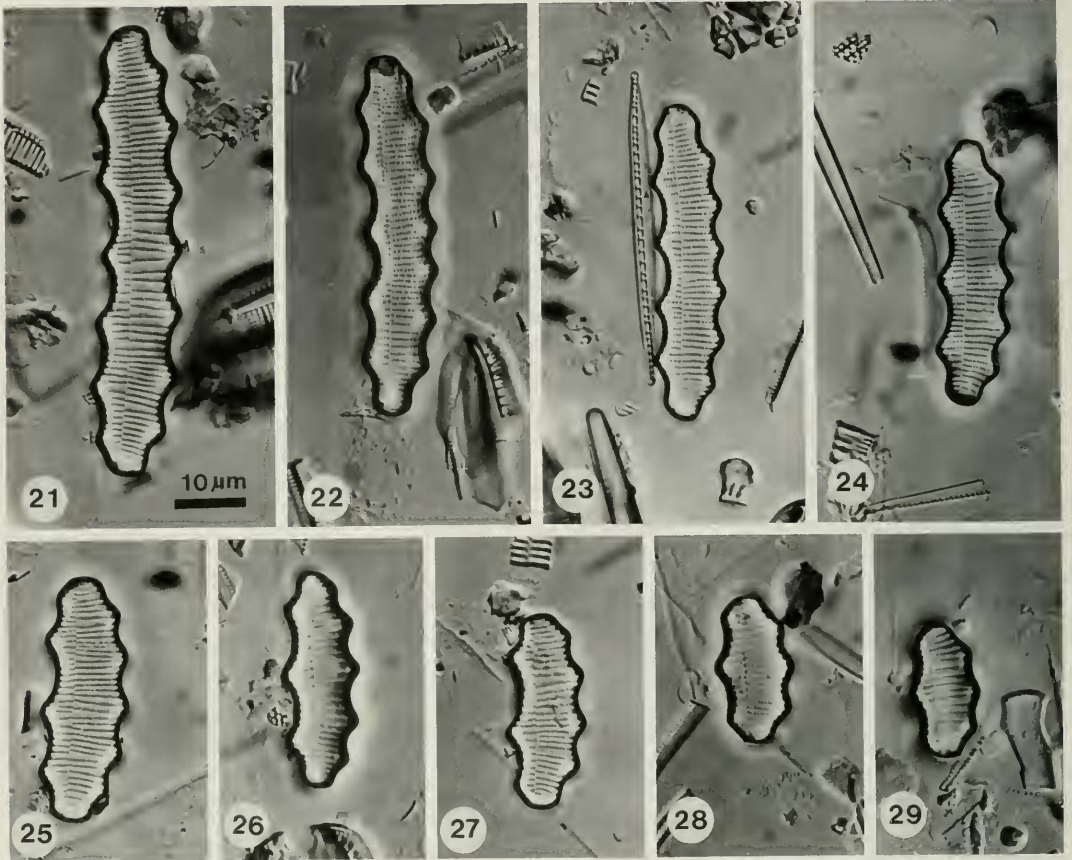
SEM OBSERVATIONS. — The external valve face is covered by striae composed of rounded areolae. Striae are interrupted towards the ventral margin on the valve face. Distinct breaks in the striae also occur around the margin of the valve. Long and short striae alternate on the valve mantle. Short raphe branches of variable shape are positioned close to the valve face-mantle junction. Spines are lacking. Internally, raphe branch length and helictoglossa size are quite variable. A rimoportula is positioned at each end of the valve. The position of the rimoportulae is variable; they may be placed at the valve terminus, at the terminus/dorsal margin junction, or along the dorsal margin.



FIGURES 11–16. *Eunotia conversa*, SEM. (11) External view. Elongate raphe sharply recurved towards valve center. Single rimoportula opening is visible. (12) External view. Valve apex with prominent raphe branch and occluded areolae. FIGS. 13–16. Internal views. (13–14) Valve apices of same valve showing elongate raphe slit bordered by thin, elevated ridge. One end (Fig. 13) has a rimoportula. Linear swelling indicates where external groove of raphe is located. (15) Proximal raphe end undifferentiated, with lack of a central nodule. (16) Center of valve showing striae interrupted in middle of valve.



FIGURES 17–20. *Peronia brasiliensis*, SEM, internal views. (17–18) Headpole (Fig. 17) and footpole (Fig. 18) of the same valve. Small raphe branches are restricted to apices. Helictoglossae are prominent. Single rimoportule is located at headpole. (19–20) Headpole (Fig. 19) and footpole (Fig. 20) of the same valve. Headpole shows striae bordering raphe to be more dense. Striae are composed of round puncta. Rimoportule is located near helictoglossa at headpole. Central nodule is lacking.

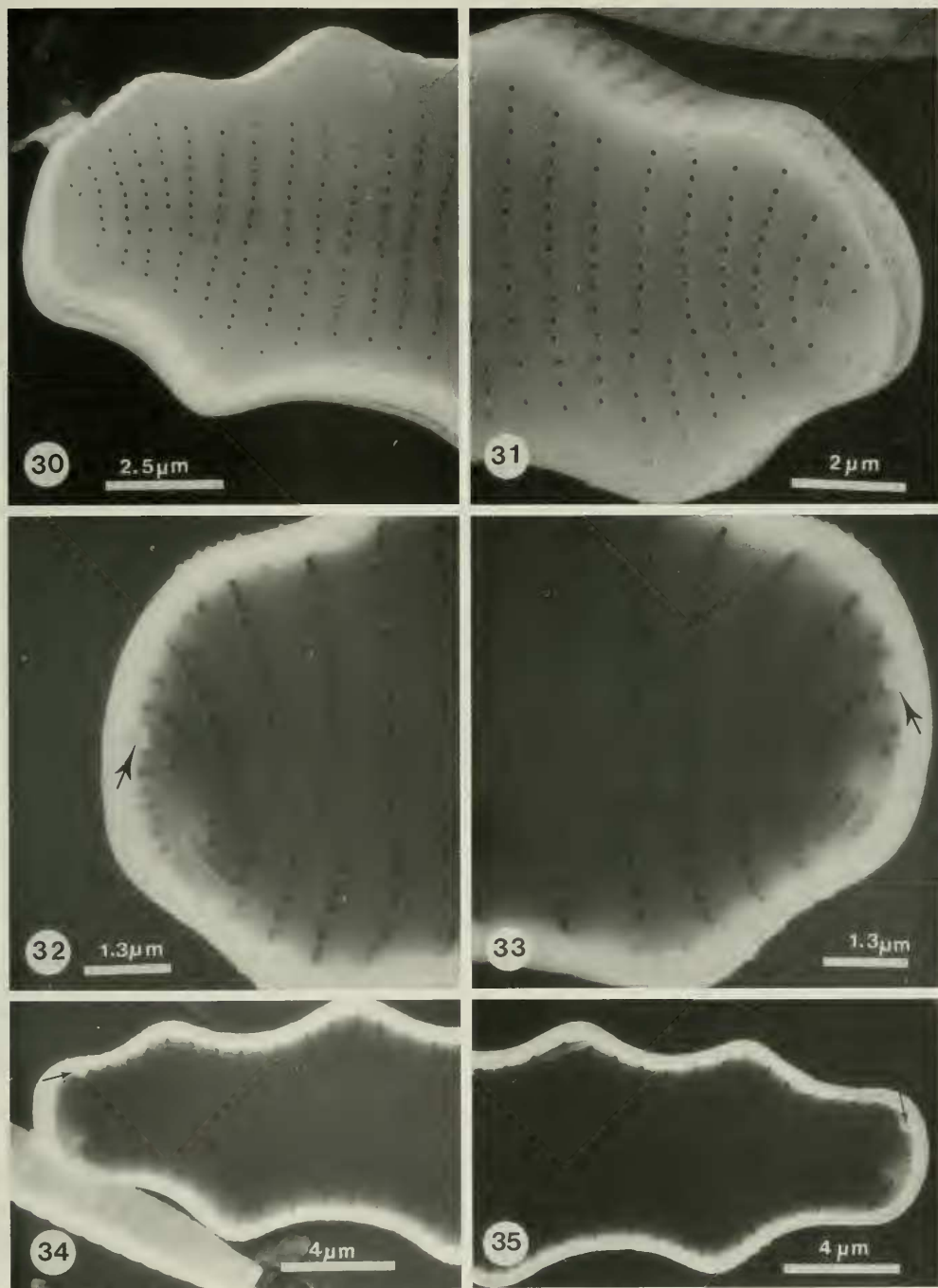


FIGURES 21–29. *Amphicampa eruca*, LM. Valve views showing variation in outline and size diminution. Scale bar is 10 μm for all figures.

DISCUSSION

Observations presented here provide additional evidence of considerable variation in raphe structure in the Eunotiaceae. This variation includes: (1) a short raphe system without a central sternum, restricted to the mantle (in *Amphicampa*); (2) a longer raphe system without a central sternum either restricted to or predominantly on the mantle (in *Eunotia* and *Actinella*; Round et al. 1990; Lange-Bertalot 1995; Kociolek et al. 1997); (3) an even longer, highly curved raphe mostly on the valve face with a central sternum (*E. conversa*, *E. curvula*, *E. synedraeformis*); and (4) a straight raphe centrally positioned on the valve face with a central sternum (*Peronia brasiliensis*).

The observations, which are based on raphe features, suggest there is variation within a genus, as well as within the family Eunotiaceae. For example, Round, Crawford, and Mann (1990:459, fig. m) illustrated what looks like a simple central nodule in a species of *Peronia*, and the lack of a central nodule in *P. brasiliensis* might suggest that *Peronia* is a non-monophyletic group. Clearly, with presence of rimoportulae being a primitive feature and presence of a raphe diagnosing the entire group of raphid diatoms, this suite of features cannot be used to diagnose the Eunotiaceae as has been done in the past (e.g., Krammer and Lange-Bertalot 1991). It is therefore possible that other rimoportula-bearing raphid diatoms do not necessarily belong to the Eunotiaceae (in either the strict or broad sense); an example of this is the genus *Eunophora*, which may be more closely aligned to *Amphora* and its relatives



FIGURES 30–35. *Amphicampa eruca*, SEM. (30–31) External views at the apices. Both figures show small raphe branches at ventral margin. Discontinuity in striae is located towards ventral margin. Striae are composed of round, unoccluded areolae. Spines are lacking. (32–33) Internal views at apices. Opposite ends of same valve showing presence of rimoportulae located on valve mantle (arrows). Raphe branches are small, with indistinct helictoglossae. (34–35) Internal views at apices. Apices of different cells showing variation in position of rimoportulae (arrows). Raphe branches and helictoglossae are noticeable.

(Vyverman et al. 1998). Presence of a central nodule in some *Peronia* species would lend support to the creation of the Peroniaceae (Round et al. 1990), since a central nodule is absent in the members of the Eunotiaceae.

Eunotia sensu stricto appears to be composed of many disparate morphological subgroups, whose relationships within the Eunotiaceae require further research. Hustedt (1926), for example, suggested the earliest branch within *Eunotia* may be composed of the bilunaris group. Krammer and Lange-Bertalot (1991) have documented with SEM the ultrastructure of *E. bilunaris* and its allies, which have raphe and valve construction similar to *E. curvula* and *E. synedraeformis*. This similarity may suggest that the bilunaris group may have evolved separately from other *Eunotia* groups. Lange-Bertalot's (1995) *E. weisingii* may be a transitional form between *E. conversa* and the bilunaris group. Variation among other *Eunotia* taxa has been documented by Krammer and Lange-Bertalot (1991) and Lange-Bertalot (1995). An implication of these observations is that *Eunotia* may not necessarily be a monophyletic group. More work is necessary to resolve the finer relationships within the Eunotiaceae.

While we may now understand the variation in raphe expression to be greater than previously appreciated, our observations do not yet speak to the question of the evolution of the raphe system from araphid ancestors. Kolbe's (1956) hypothesis of the earliest raphid-bearing diatom being similar to *Amphicampa* (supported by the work of Mann 1984), then evolving through forms with progressively longer raphe systems until the naviculoid type is reached, is consistent with our observations. However, equally plausible is the idea that the earliest raphe system was fully developed (similar to that in *Peronia brasiliensis*, Hustedt 1952; see also Hustedt 1935), and that the rest of the eunotioid forms represent degeneration, with *Amphicampa* showing the greatest expression of raphe reduction. Kociolek and Rhode (1998) have suggested raphe reduction occurred elsewhere within the raphidiod line. In the *Actinella* lineage from Madagascar, a series of raphe types were observed, and suggested to represent reduction of a typical *Actinella* raphe system to a simple slit without a helictoglossa (Kociolek and Rhode 1998). Taxa such as *Falcula* Voigt, shown to have a slit positioned close to the ventral margin may reflect a degenerated (but derived) condition of the raphe, rather than a primitive raphe condition, as argued by Geissler and Gerloff (1963). Given the diversity within *Amphicampa* (Ehrenberg 1854) and the closely allied genera *Ophidocampa* and *Heterocampa* (Ehrenberg 1870), both of which have not been investigated since the taxa were described originally, the relationship of *Amphicampa* and its close allies within the Eunotiaceae needs further investigation. Also, examination of original Ehrenberg material to confirm the identity of *Amphicampa eruca* is crucial to confirming the present results.

Further observations on other naviculoid forms, including *Rouxia*, *Amphiptera* and *Frustulia* among others, as well as other raphe- and rimoportula-bearing taxa (such as *Eunophora*) will be critical in developing a formal phylogenetic hypothesis on the origin and early evolution of the raphe system.

ACKNOWLEDGMENTS

Sarah Spaulding, CAS, graciously provided technical assistance. Discussions with participants of the XIV North American Diatom Symposium (1997) about these findings were particularly helpful. Reviews of this manuscript by S. Spaulding, E. Fourtanier, R. Jahn, D. Williams and the research group in the laboratory of E. F. Stoermer were also helpful.

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